F7_12 = F7_7 + F8_8 + F9_9 + F10_10 + F11_11 + F12_12
F7_13 = F7_7 + F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13
F7_14 = F7_7 + F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14
F7_15 = F7_7 + F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15
F7_16 = F7_7 + F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16
F7_17 = F7_7 + F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17
F7_18 = F7_7 + F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17 + F18_18
F8_8 = F8_8
F8_9 = F8_8 + F9_9
F8_10 = F8_8 + F9_9 + F10_10
F8_11 = F8_8 + F9_9 + F10_10 + F11_11
F8_12 = F8_8 + F9_9 + F10_10 + F11_11 + F12_12
F8_13 = F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13
F8_14 = F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14
F8_15 = F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15
F8_16 = F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16
F8_17 = F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17
F8_18 = F8_8 + F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17 + F18_18
F9_9 = F9_9
F9_10 = F9_9 + F10_10
F9_11 = F9_9 + F10_10 + F11_11
F9_12 = F9_9 + F10_10 + F11_11 + F12_12
F9_13 = F9_9 + F10_10 + F11_11 + F12_12 + F13_13
F9_14 = F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14
F9_15 = F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15
F9_16 = F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16
F9_17 = F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17
F9_18 = F9_9 + F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17 + F18_18
F10_10 = F10_10
F10_11 = F10_10 + F11_11
F10_12 = F10_10 + F11_11 + F12_12
F10_13 = F10_10 + F11_11 + F12_12 + F13_13
F10_14 = F10_10 + F11_11 + F12_12 + F13_13 + F14_14
F10_15 = F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15
F10_16 = F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16
F10_17 = F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17
F10_18 = F10_10 + F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17 + F18_18
F11_11 = F11_11
F11_12 = F11_11 + F12_12
F11_13 = F11_11 + F12_12 + F13_13
F11_14 = F11_11 + F12_12 + F13_13 + F14_14
F11_15 = F11_11 + F12_12 + F13_13 + F14_14 + F15_15
F11_16 = F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16
F11_17 = F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17
F11_18 = F11_11 + F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17 + F18_18
F12_12 = F12_12
F12_13 = F12_12 + F13_13
F12_14 = F12_12 + F13_13 + F14_14
F12_15 = F12_12 + F13_13 + F14_14 + F15_15
F12_16 = F12_12 + F13_13 + F14_14 + F15_15 + F16_16
F12_17 = F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17
F12_18 = F12_12 + F13_13 + F14_14 + F15_15 + F16_16 + F17_17 + F18_18
F13_13 = F13_13
F13_14 = F13_13 + F14_14
F13_15 = F13_13 + F14_14 + F15_15
F13_16 = F13_13 + F14_14 + F15_15 + F16_16
F13_17 = F13_13 + F14_14 + F15_15 + F16_16 + F17_17
F13_18 = F13_13 + F14_14 + F15_15 + F16_16 + F17_17 + F18_18
F14_14 = F14_14
F14_15 = F14_14 + F15_15
F14_16 = F14_14 + F15_15 + F16_16
F14_17 = F14_14 + F15_15 + F16_16 + F17_17
F14_18 = F14_14 + F15_15 + F16_16 + F17_17 + F18_18

F15_15 = F15_15
F15_16 = F15_15 + F16_16
F15_17 = F15_15 + F16_16 + F17_17
F15_18 = F15_15 + F16_16 + F17_17 + F18_18
F16_16 = F16_16
F16_17 = F16_16 + F17_17
F16_18 = F16_16 + F17_17 + F18_18
F17_17 = F17_17
F17_18 = F17_17 + F18_18
F18_18 = F18_18

Once the sequence of each pre-ligated fragment is determined, the system begins to estimate the portions of each pre-ligated sequence to be used to generate the desired PDF. As discussed above, the ligation reaction for a sequence having 18 fragments preferably takes place as 18 separate reactions. Thus, the system generates a starting set of ligation reactions for each of the 18 separate ligations. It should be noted that each ligation step uses progressively fewer of the pre-ligated molecules. This is due to the fact that, for example, the third step of the ligation reaction would not require pre-ligated fragments starting with fragment 1 "F1" or fragment 2 (F2) since these fragments have already been ligated to other fragments by the third step in the ligation. At step three, there should only ligation of fragments that bind to the third fragment from each parent.

For example, the following are exemplary ligation reactions that take place within the memory of the computer system.

Number of Ligation Steps: 18

Simulated Ligation volume of each step (ul): 100

Ligation Step #1	Ligation Step #2	Ligation Step #3	Ligation Step #4	Ligation Step #5
0.6 ul of F1_1	0.7 ul of F2_2	0.7 ul of F3_3	0.8 ul of F4_4	1.0 ul of F5_5
1.2 ul of F1_2	1.3 ul of F2_3	1.5 ul of F3_4	1.7 ul of F4_5	1.9 ul of F5_6
1.8 ul of F1_3	2.0 ul of F2_4	2.2 ul of F3_5	2.5 ul of F4_6	2.9 ul of F5_7
2.3 ul of F1_4	2.6 ul of F2_5	2.9 ul of F3_6	3.3 ul of F4_7	3.8 ul of F5_8
2.9 ul of F1_5	3.3 ul of F2_6	3.7 ul of F3_7	4.2 ul of F4_8	4.8 ul of F5_9
3.5 ul of F1_6	3.9 ul of F2_7	4.4 ul of F3_8	5.0 ul of F4_9	5.7 ul of F5_10
4.1 ul of F1_7	4.6 ul of F2_8	5.1 ul of F3_9	5.8 ul of F4_10	6.7 ul of F5_11
4.7 ul of F1_8	5.2 ul of F2_9	5.9 ul of F3_10	6.7 ul of F4_11	7.6 ul of F5_12
5.3 ul of F1_9	5.9 ul of F2_10	6.6 ul of F3_11	7.5 ul of F4_12	8.6 ul of F5_13
5.8 ul of F1_10	6.5 ul of F2_11	7.4 ul of F3_12	8.3 ul of F4_13	9.5 ul of F5_14
6.4 ul of F1_11	7.2 ul of F2_12	8.1 ul of F3_13	9.2 ul of F4_14	10.5 ul of F5_15

7.0 ul of F1_12 7.6 ul of F1_13 8.2 ul of F1_14 8.8 ul of F1_15 9.4 ul of F1_16 9.9 ul of F1_17 10.5 ul of F1_18	7.8 ul of F2_13 8.5 ul of F2_14 9.2 ul of F2_15 9.8 ul of F2_16 10.5 ul of F2_17 11.1 ul of F2_18	8.8 ul of F3_14 9.6 ul of F3_15 10.3 ul of F3_16 11.0 ul of F3_17 11.8 ul of F3_18	10.0 ul of F4_15 10.8 ul of F4_16 11.7 ul of F4_17 12.5 ul of F4_18	11.4 ul of F5_16 12.4 ul of F5_17 13.3 ul of F5_18
Ligation Step #6:	**Ligation Step #7**	**Ligation Step #8**	**Ligation Step #9**	**Ligation Step #10**
1.1 ul of F6_6 2.2 ul of F6_7 3.3 ul of F6_8 4.4 ul of F6_9 5.5 ul of F6_10 6.6 ul of F6_11 7.7 ul of F6_12 8.8 ul of F6_13 9.9 ul of F6_14 11.0 ul of F6_15 12.1 ul of F6_16 13.2 ul of F6_17 14.3 ul of F6_18	1.3 ul of F7_7 2.6 ul of F7_8 3.8 ul of F7_9 5.1 ul of F7_10 6.4 ul of F7_11 7.7 ul of F7_12 9.0 ul of F7_13 10.3 ul of F7_14 11.5 ul of F7_15 12.8 ul of F7_16 14.1 ul of F7_17 15.4 ul of F7_18	1.5 ul of F8_8 3.0 ul of F8_9 4.5 ul of F8_10 6.1 ul of F8_11 7.6 ul of F8_12 9.1 ul of F8_13 10.6 ul of F8_14 12.1 ul of F8_15 1 3.6 ul of F8_16 15.2 ul of F8_17 16.7 ul of F8_18	1.8 ul of F9_9 3.6 ul of F9_10 5.5 ul of F9_11 7.3 ul of F9_12 9.1 ul of F9_13 10.9 ul of F9_14 12.7 ul of F9_15 14.5 ul of F9_16 16.4 ul of F9_17 18.2 ul of F9_18	2.2 ul of F10_10 4.4 ul of F10_11 6.7 ul of F10_12 8.9 ul of F10_13 11.1 ul of F10_14 13.3 ul of F10_15 15.6 ul of F10_16 17.8 ul of F10_17 20.0 ul of F10_18
Ligation Step #11	**Ligation Step #12**	**Ligation Step #13**	**Ligation Step #14**	**Ligation Step #15**
2.8 ul of F11_11 5.6 ul of F11_12 8.3 ul of F11_13 11.1 ul of F11_14 13.9 ul of F11_15 16.7 ul of F11_16 19.4 ul of F11_17 22.2 ul of F11_18	3.6 ul of F12_12 7.1 ul of F12_13 10.7 ul of F12_14 14.3 ul of F12_15 17.9 ul of F12_16 21.4 ul of F12_17 25.0 ul of F12_18	4.8 ul of F13_13 9.5 ul of F13_14 14.3 ul of F13_15 19.0 ul of F13_16 23.8 ul of F13_17 28.6 ul of F13_18	6.7 ul of F14_14 13.3 ul of F14_15 20.0 ul of F14_16 26.7 ul of F14_17 33.3 ul of F14_18	10.0 ul of F15_15 20.0 ul of F15_16 30.0 ul of F15_17 40.0 ul of F15_18
Ligation Step #16	**Ligation Step #17**	**Ligation Step #18**		
16.7 ul of F16_16 33.3 ul of F16_17	33.3 ul of F17_17 66.7 ul of F17_18	100.0 ul of F18_18		

50.0 ul of F16_18				

Carrying out the preceding ligation reactions results in a calculated PDF. Thus, the system can then adjust the volumes of each pre-ligated fragment during a further round of simulated reassembly until the PDF matches the desired probability function. The majority of progeny molecules only have one or two crossover events. Adjusting the quantities of the ligation reactions, as shown below will skew the PDF so that it moves towards progeny molecules having more crossover events.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned as well as those inherent therein. The methods described herein are presently representative of exemplary aspects and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention and are defined by the scope of the claims.

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WHAT IS CLAIMED IS:

1. A method for producing a library of nucleic acids encoding a plurality of modified antigen binding sites, wherein the modified antigen binding sites are derived from a first nucleic acid comprising a sequence encoding a first antigen binding site, the method comprising:

(a) providing a first nucleic acid encoding a first antigen binding site;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

(c) using the set of mutagenic oligonucleotides to generate a set of antigen binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized,

thereby producing a library of nucleic acids encoding a plurality of modified antigen binding sites.

2. The method of claim 1, wherein step (b) provides a set of mutagenic oligonucleotides that encode all nineteen naturally-occurring amino acid variants for each targeted codon, thereby generating all 19 possible natural amino acid changes at each amino acid codon mutagenized.

3. The method of claim 1, further comprising expressing the set of variant antigen binding site-encoding nucleic acids such that antigen binding site-encoding polypeptides encoded by the variant nucleic acids are expressed.

4. The method of claim 1, wherein the set of mutagenic oligonucleotides comprises a 19-fold degenerate mutagenic oligonucleotide for each codon to be mutagenized, wherein each of the 19-fold degenerate mutagenic oligonucleotides comprises a homologous first sequence and a degenerate triplet second sequence.

5. The method of claim 1, wherein the antigen binding site comprises a single stranded antigen binding polypeptide, a Fab fragment, an Fc fragment, a Fd fragment, a $F(ab')_2$ fragment, a Fv fragment or a complementarity determining region (CDR).

6. The method of claim 5, wherein the antigen binding site polypeptide further comprises an antibody polypeptide.

7. The method of claim 1, wherein the antigen binding site polypeptide further comprises an antigen binding site of a T cell receptor (TCR).

8. The method of claim 7, wherein the antigen binding site polypeptide further comprises a T cell receptor (TCR).

9. The method of claim 1, wherein the antigen binding site polypeptide further comprises an antigen binding site of a major histocompatibility complex (MHC) molecule.

10. The method of claim 9, wherein the antigen binding site polypeptide further comprises a major histocompatibility complex (MHC) molecule.

11. The method of claim 10, wherein the major histocompatibility complex (MHC) molecule comprises a Class I molecule.

12. The method of claim 10, wherein the major histocompatibility complex (MHC) molecule comprises a Class II molecule.

13. The method of claim 1, wherein the nucleic acid of step (a) is derived from a nucleic acid encoding a mammalian polypeptide.

14. The method of claim 13, wherein the mammalian polypeptide comprises a human polypeptide.

15. The method of claim 13, wherein the mammalian polypeptide is selected from the group consisting of an antibody, a T cell receptor, a Class I MHC molecule and a Class II MHC molecule.

16. The method of claim 1, wherein the nucleic acid of step (a) is derived from a human nucleic acid encoding an antigen binding site.

17. The method of claim 16, wherein the nucleic acid of step (a) is derived from a phage comprising a human nucleic acid sequence encoding an antigen binding site, wherein the phage expresses the antigen binding site.

18. The method of claim 16, wherein the nucleic acid of step (a) is derived from a non-human mammal comprising a human nucleic acid sequence encoding an antigen binding site, wherein the non-human mammal expresses the antigen binding site.

19. The method of claim 18, wherein the non-human mammal is a transgenic non-human mammal.

20. The method of claim 19, wherein the transgenic non-human mammal is a mouse.

21. The method of claim 1, wherein at least two amino acid codons in the antigen binding site are mutagenized.

22. The method of claim 21, wherein all the amino acid codons in the antigen binding site are mutagenized.

23. The method of claim 6, wherein all the amino acid codons in the antibody polypeptide are mutagenized.

24. The method of claim 8, wherein all the amino acid codons in the T cell receptor (TCR) are mutagenized.

25. The method of claim 10, wherein all the amino acid codons in the MHC molecule are mutagenized.

26. The method of claim 1, wherein a degenerate mutagenic oligonucleotide comprises a first homologous sequence, a degenerate triplet second sequence, and a third homologous sequence.

27. The method of claim 1, wherein each degenerate oligonucleotide comprises a first homologous sequence, a plurality of degenerate triplets second sequences, and a third homologous sequence.

28. The method of claim 3, further comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen.

29. The method of claim 28, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen capable of being specifically bound by the first antigen binding site polypeptide.

30. The method of claim 29, comprising identifying an antigen binding site variant by its increased antigen binding affinity or antigen binding specificity as compared to the affinity or specificity of the first antigen binding site to the antigen.

31. The method of claim 29, comprising identifying an antigen binding site variant by its decreased antigen binding affinity or antigen binding specificity as compared to the affinity or specificity of the first antigen binding site to the antigen.

32. The method of claim 1, further comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system.

33. The method of claim 1, further comprising mutagenizing the first nucleic acid of step (a) by a method comprising a synthetic ligation reassembly.

34. The method of claim 3, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising expression of the expressed antigen binding site polypeptide in a solid phase.

35. The method of claim 34, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising a capillary array.

36. The method of claim 34, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising a double-orificed container.

37. The method of claim 36, wherein the double-orificed container comprises a double-orificed capillary array.

38. The method of claim 37, wherein the double-orificed capillary array is a GIGAMATRIX™ capillary array.

39. The method of claim 34, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising use of an ELISA.

40. The method of claim 3, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising phage display of the antigen binding site polypeptide.

41. The method of claim 3, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising expression of the expressed antigen binding site polypeptide in a liquid phase.

42. The method of claim 3, comprising screening the expressed antigen binding site polypeptide for its ability to specifically bind an antigen by a method comprising ribosome display of the antigen binding site polypeptide.

43. The method of claim 1, wherein the set of progeny antigen binding site-encoding variant nucleic acids is generated by amplifying the nucleic acid of step (a) by a polymerase-based amplification using a plurality of oligonucleotides.

44. The method of claim 43, wherein the amplification comprises a polymerase chain reaction (PCR).

45. A library of nucleic acids encoding a plurality of modified antigen binding sites, wherein the modified antigen binding sites are derived from a first nucleic acid comprising a sequence encoding a first antigen binding site, made by a method comprising the following steps:

(a) providing a first nucleic acid encoding a first antigen binding site;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

(c) using the set of mutagenic oligonucleotides to generate a set of antigen binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized,

thereby producing a library of nucleic acids encoding a plurality of modified antigen binding sites.

46. A method for producing from a library of variant antibodies from a template antibody, the method comprising:

(a) providing a first nucleic acid encoding the template antibody;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

c) using the set of mutagenic oligonucleotides to generate a set of antibody-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized,

thereby producing a library of nucleic acids encoding a plurality of variant antibodies.

47. The method of claim 46, wherein step (b) provides a set of mutagenic oligonucleotides that encode all nineteen naturally-occurring amino acid variants for each targeted codon, thereby generating all 19 possible natural amino acid changes at each amino acid codon mutagenized.

48. The method of claim 46, wherein the antibody is selected from the group consisting of polypeptides comprising a Fab fragment, an Fd fragment, an Fc fragment, a F(ab')2 fragment, a Fv fragment and a complementarity determining region (CDR).

49. The method of claim 46, wherein the plurality of oligonucleotides comprises a degenerate oligonucleotide for each codon to be mutagenized, wherein each of the degenerate oligonucleotides comprises a homologous first sequence and a degenerate triplet second sequence.

50. The method of claim 46, wherein the set of progeny polynucleotides encoding antibodies is generated by amplifying the nucleic acid of step (a) using a plurality of oligonucleotides.

51. A library of variant antibodies derived from a template antibody made by a method comprising the following steps:

(a) providing a first nucleic acid encoding the template antibody;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

c) using the set of mutagenic oligonucleotides to generate a set of antibody-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized,

thereby producing a library of nucleic acids encoding a plurality of variant antibodies.

52. A method for producing from a library of variant T cell receptors (TCRs) from a template T cell receptor (TCR), the method comprising:

(a) providing a first nucleic acid encoding the template T cell receptor;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

c) using the set of mutagenic oligonucleotides to generate a set of T cell receptor (TCR)-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized,

thereby producing a library of nucleic acids encoding a plurality of variant T cell receptors (TCRs).

53. A library of variant T cell receptors (TCRs) derived from a template T cell receptor (TCR) made by a method comprising the following steps:

(a) providing a first nucleic acid encoding the template T cell receptor;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

c) using the set of mutagenic oligonucleotides to generate a set of T cell receptor (TCR)-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized,

thereby producing a library of nucleic acids encoding a plurality of variant T cell receptors (TCRs).

54. A method for producing from a library of variant major histocompatibility complex (MHC) molecules from a template major histocompatibility complex (MHC) molecule, the method comprising:

(a) providing a first nucleic acid encoding the template major histocompatibility complex (MHC) molecule;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

c) using the set of mutagenic oligonucleotides to generate a set of major histocompatibility complex (MHC) molecule-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized,

thereby producing a library of nucleic acids encoding a plurality of variant major histocompatibility complex (MHC) molecules.

55. A library of variant major histocompatibility complex (MHC) molecules derived from a template major histocompatibility complex (MHC) molecule made by a method comprising the following steps:

(a) providing a first nucleic acid encoding the template major histocompatibility complex (MHC) molecule;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

c) using the set of mutagenic oligonucleotides to generate a set of major histocompatibility complex (MHC) molecule-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized,

thereby producing a library of nucleic acids encoding a plurality of variant major histocompatibility complex (MHC) molecules.

56. A method of making a set of nucleic acids encoding a set of antigen binding site variants comprising the steps of:

(a) providing a template nucleic acid encoding an antigen-binding polypeptide;

(b) providing a plurality of oligonucleotides that encode all nineteen naturally-occurring amino acid variants at a single amino acid residue of the antigen-binding polypeptide; and,

(c) generating a set of progeny antigen binding site-encoding variant nucleic acids encoding a non-stochastic range of single amino acid substitutions at each amino acid codon that was mutagenized, whereby all 19 possible natural amino acid changes are generated at each amino acid codon mutagenized,

thereby making a set of nucleic acids encoding a set of antigen binding site variants.

57. The method of claim 56, further comprising expressing the set of progeny antigen binding site-encoding polynucleotides such that antigen binding site-encoding polypeptides encoded by the progeny polynucleotides are expressed.

58. The method of claim 56, wherein the plurality of oligonucleotides comprises a set of degenerate oligonucleotides and each of the degenerate oligonucleotides comprises a homologous first sequence and a degenerate triplet second sequence.

59. The method of claim 56, wherein the antigen binding site-encoding polypeptide comprises a single stranded antigen binding polypeptide.

60. The method of claim 56, wherein the antigen binding site-encoding polypeptide comprises an antibody polypeptide.

61. The method of claim 56, wherein the antigen binding site-encoding polypeptide comprises an antigen binding site of a T cell receptor (TCR).

62. The method of claim 61, wherein the antigen binding site-encoding polypeptide further comprises a T cell receptor (TCR).

63. The method of claim 56, wherein the antigen binding site-encoding polypeptide comprises an antigen binding site of a major histocompatibility complex (MHC) molecule.

64. The method of claim 63, wherein the antigen binding site-encoding polypeptide further comprises a major histocompatibility complex (MHC) molecule.

65. The method of claim 56, wherein the nucleic acid of step (a) is derived from a nucleic acid encoding a mammalian antibody polypeptide.

66. The method of claim 65, wherein the nucleic acid of step (a) is derived from a human nucleic acid.

67. The method of claim 56, wherein at least two amino acid codons in the antigen binding site are mutagenized and a set of degenerate oligonucleotides that encode all

nineteen naturally-occurring amino acid variants are provided for each amino acid codon mutagenized.

68. The method of claim 56, wherein all the amino acid codons in the antigen binding site are mutagenized and a set of degenerate oligonucleotides that encode all nineteen naturally-occurring amino acid variants are provided for each amino acid codon mutagenized.

69. The method of claim 60, wherein all the amino acid codons in the antibody polypeptide are mutagenized.

70. The method of claim 61, wherein all the amino acid codons in the antigen binding site of the T cell receptor (TCR) are mutagenized.

71. The method of claim 63, wherein all the amino acid codons in the antigen binding site of the major histocompatibility complex (MHC) molecule are mutagenized.

72. The method of claim 56, wherein a degenerate oligonucleotide comprises a first homologous sequence, a degenerate triplet second sequence, and a homologous third sequence.

73. The method of claim 56, wherein each degenerate oligonucleotide comprises a first homologous sequence, a degenerate triplet second sequence, and a homologous third sequence.

74. The method of claim 57, further comprising screening an expressed antigen binding site-encoding polypeptide for its ability to specifically bind an antigen.

75. The method of claim 57, comprising screening the expressed antigen binding site-encoding polypeptide for its ability to specifically bind an antigen capable of being specifically bound by the first antigen binding site.

76. The method of claim 75, comprising identifying an antigen binding site variant by its increased antigen binding affinity or antigen binding specificity to the antigen as compared to the affinity or specificity of the antigen binding site encoded by the nucleic acid of step (a).

77. The method of claim 56, further comprising mutagenizing the template nucleic acid by a method comprising an optimized directed evolution system.

78. The method of claim 56, further comprising mutagenizing the template nucleic acid by a method comprising a synthetic ligation reassembly.

79. The method of claim 56, comprising screening the expressed antigen binding site-encoding polypeptide for its ability to specifically bind an antigen by a method comprising a capillary array.

80. The method of claim 56, comprising screening the expressed antigen binding site-encoding polypeptide for its ability to specifically bind an antigen by an ELISA.

81. The method of claim 56, wherein the set of variant nucleic acids is generated by performing amplification reactions on the nucleic acid of step (a) using the set of oligonucleotides to generate a set of variant nucleic acids encoding nineteen amino acid substitution variants at a single amino acid residue of the antigen-binding polypeptide.

82. The method of claim 81, wherein the amplification comprises a polymerase-based amplification.

83. The method of claim 82, wherein polymerase-based amplification comprises a polymerase chain reaction (PCR).

84. The method of claim 56, wherein the set of variant nucleic acids comprises 10^{10} members.

85. The method of claim 56, wherein the set of variant nucleic acids comprises 10^5 members.

86. . The method of claim 56, wherein the set of variant nucleic acids comprises 10^3 members.

87. A method of making a set of antibody variants comprising the steps of:

(a) providing a nucleic acid encoding an antibody;

(b) providing a plurality of oligonucleotides;

(c) generating a non-stochastic range of single amino acid substitutions at each amino acid codon, whereby all 19 possible natural amino acid changes are generated at each amino acid codon mutagenized, thereby generating a set of variant nucleic acids; and,

(d) expressing the set of variant nucleic acids such that the antibody variants encoded by the variant nucleic acids are expressed.

88. The method of claim 87, wherein the antibody is selected from the group consisting of polypeptides comprising a Fab fragment, a Fd fragment, an Fc fragment, a F(ab')2 fragment, a Fv fragment and a complementarity determining region (CDR).

89. The method of claim 87, wherein the plurality of oligonucleotides comprises a set of degenerate oligonucleotides that encode all nineteen naturally-occurring amino acid variants at a single amino acid residue of the antibody, wherein each of the degenerate oligonucleotides comprises a homologous first sequence and a degenerate triplet second sequence.

90. The method of claim 87, wherein generating a non-stochastic range of single amino acid substitutions comprises performing amplification reactions on the nucleic acid of step (a) using the set of oligonucleotides to generate a set of variant nucleic acids encoding nineteen amino acid substitution variants at a single amino acid residue of the antibody.

91. A method of identifying a variant of an antigen binding site comprising the steps of:

(a) providing a nucleic acid encoding an antigen binding site;

(b) providing a set of oligonucleotides that encode all nineteen naturally-occurring amino acid variants at all residues of the antigen- binding site;

(c) incorporating the sequence of the oligonucleotides of step (b) into the nucleic acid of step (a) to generate a set of variant nucleic acids encoding nineteen amino acid substitution variants at each residue of the antigen binding site;

(d) expressing each of the variant nucleic acids as polypeptides and measuring the variant's affinity to the antigen; and,

(e) identifying a variant of the antigen binding site by its increased or decreased antigen binding specificity as compared to the antigen binding affinity of the antigen binding site encoded by the nucleic acid of step (a).

92. The method of claim 91, wherein the variant nucleic acids are expressed using *in vitro* transcription/translation.

93. The method of claim 91, wherein the variant nucleic acids are expressed using phage display.

94. The method of claim 91, wherein the variant nucleic acids are expressed using ribosome display.

95. The method of claim 91, wherein the variant nucleic acids are expressed using a double orificed container.

96. The method of claim 95, wherein the variant nucleic acids are expressed using a double orificed capillary array.

97. The method of claim 91, wherein the set of oligonucleotides comprises a set of degenerate oligonucleotides that encode all nineteen naturally-occurring amino acid variants at a single amino acid residue of the antibody, wherein each of the degenerate oligonucleotides comprises a homologous first sequence and a degenerate triplet second sequence.

98. The method of claim 91, wherein the antigen binding site comprises an antibody.

99. The method of claim 98, wherein the antibody is selected from the group consisting of polypeptides comprising a Fab fragment, an Fd fragment, an Fc fragment, a F(ab')2 fragment, a Fv fragment and a complementarity determining region (CDR).

100. The method of claim 91, wherein the antigen binding site comprises an antigen binding site of a T cell receptor.

101. The method of claim 91, wherein the antigen binding site comprises an antigen binding site of a major histocompatibility complex molecule.

102. The method of claim 91, wherein incorporating the sequence of the oligonucleotides of step (b) into the nucleic acid of step (a) is accomplished by an amplification reaction using the oligonucleotides as primers.

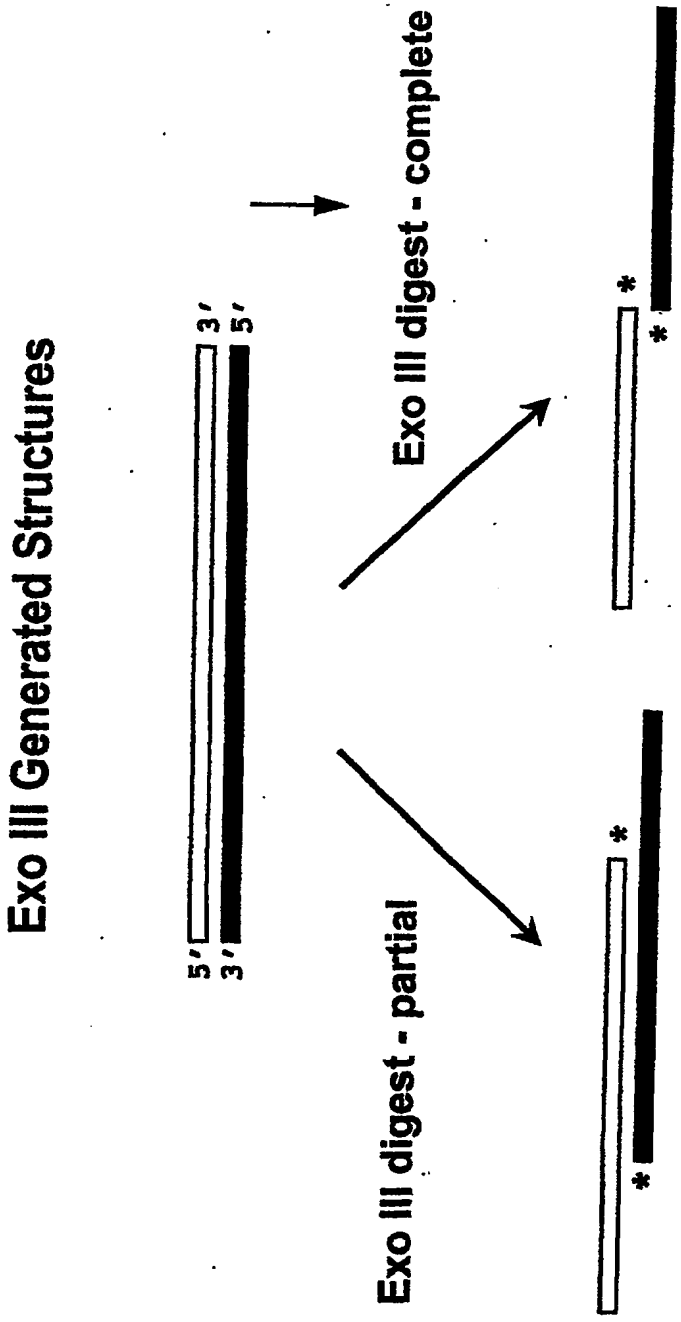

Exo III Generated Structures
3'
5'
5'
3'
Exo III digest - complete
Exo III digest - partial
*
*
*
*

Figure 1

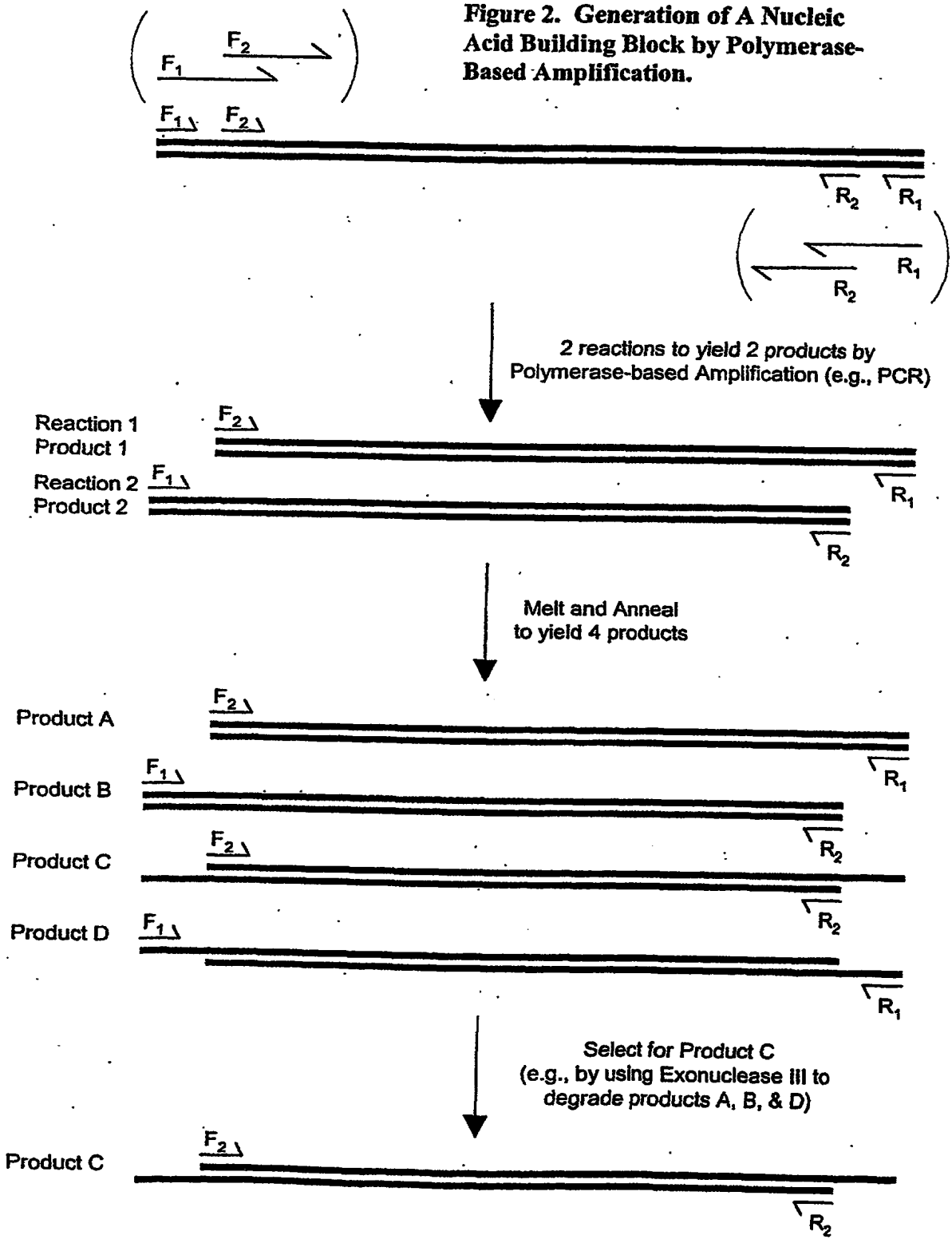
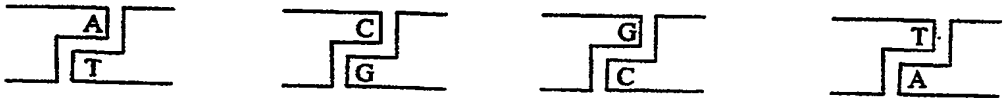


Figure 2. Generation of A Nucleic Acid Building Block by Polymerase-Based Amplification.

FIGURE 3. Unique Overhangs And Unique Couplings.

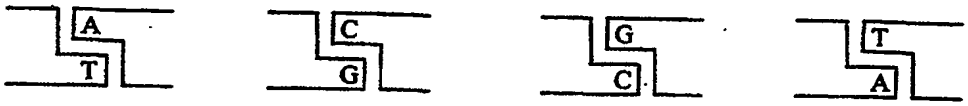
The number of unique overhangs of each size (e.g. the total number of unique overhangs composed of 1 or 2 or 3, etc. nucleotides) exceeds the number of unique couplings that can result from the use of all the unique overhangs of that size. For example, the total number of unique couplings that can be made using all the 8 unique single-nucleotide 3' overhangs and single-nucleotide 5' overhangs is 4.

PANEL A. 4 unique single-nucleotide 3' overhangs are possible (i.e., A, C, G, & T). For each of these there is a complementary 3' overhang with which it can pair (i.e., T, G, C, & A, respectively), as shown.



PANEL B. However, the number of unique single-nucleotide 3' overhangs is greater than the number of unique couplings. Thus, only 2 intrinsically unique couplings exist using single-nucleotide 3' overhangs as shown.

PANEL C. Likewise, 4 unique-single nucleotide 5' overhangs are possible (i.e., A, C, G, & T). For each of these there is a complementary 5' overhang with which it can pair (i.e., T, G, C, & A, respectively), as shown.

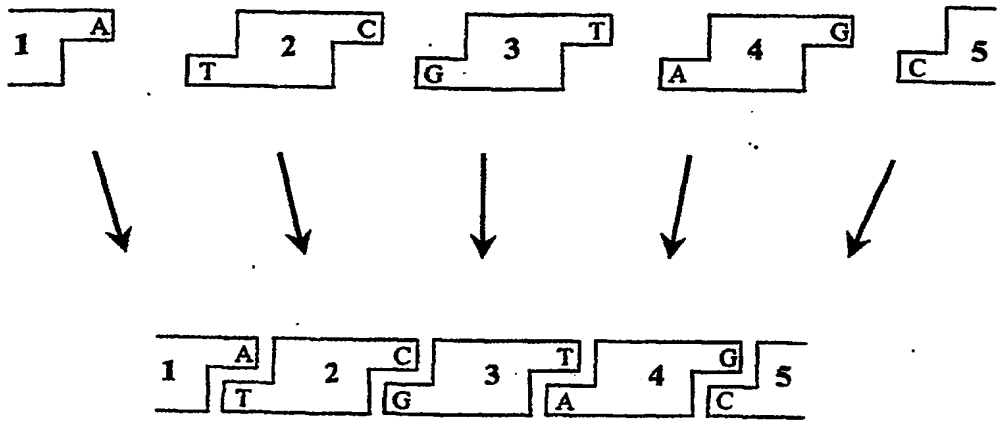


PANEL D. However, the number of unique single-nucleotide 5' overhangs is greater than the number of unique couplings. Thus, only 2 intrinsically unique couplings exist using single-nucleotide 5' overhangs as shown.

FIGURE 4. Unique Overall Assembly Order Achieved by Sequentially Coupling the Building Blocks

Awareness of the degeneracy (between the number of unique overhangs and the number of unique couplings) is important in order to avoid the production of degeneracy in the overall assembly order of the finalized nucleic acid. However, a unique overall assembly order can also be achieved - despite the use of non-unique couplings - by using building blocks having distinct combinations of couplings, and/or by stepping the assembly of the building blocks in a deliberately chosen sequence.

PANEL A. For example, one could attempt to assemble the following nucleic acid product using the 5 nucleic acid building blocks as shown.



PANEL B. However, degeneracy in the overall assembly order of the 5 nucleic acid building blocks would be present if the assembly process were carried out in one step. For example, building block #2 and building block #3 could both couple to building block #1 as shown.

FIGURE 4 cont.

PANEL C. However, a unique overall assembly order could be achieved by sequentially coupling the building blocks in 2 steps (rather than all at once) as shown.

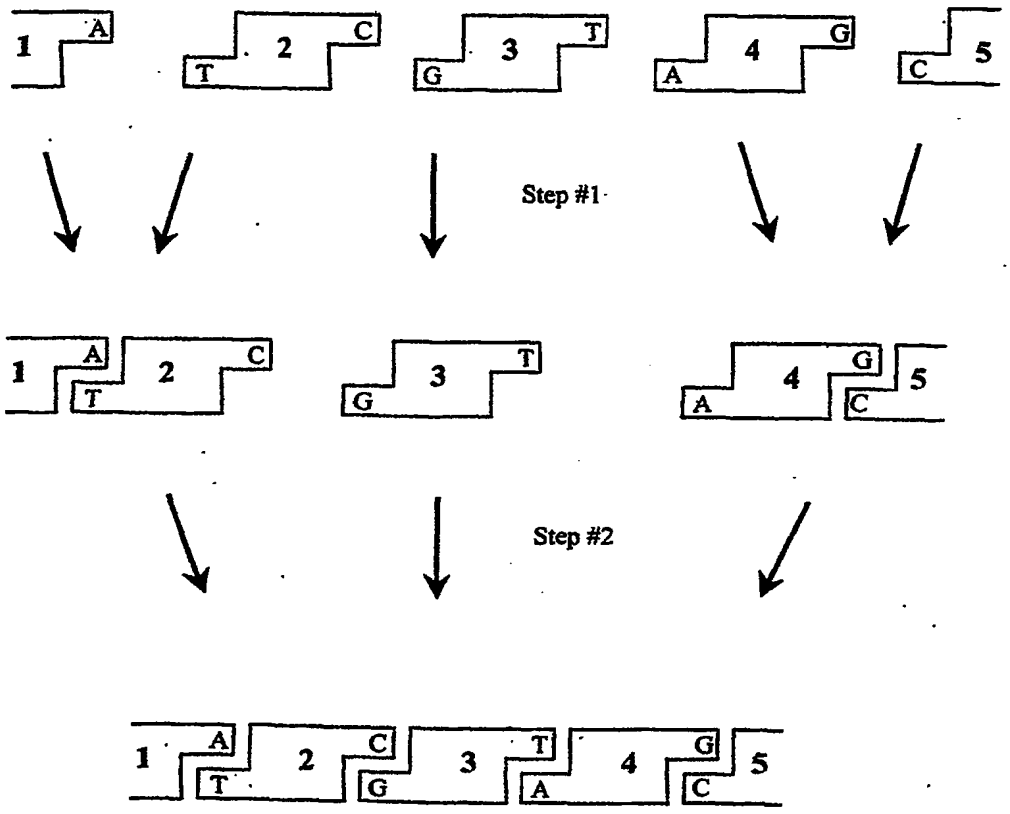
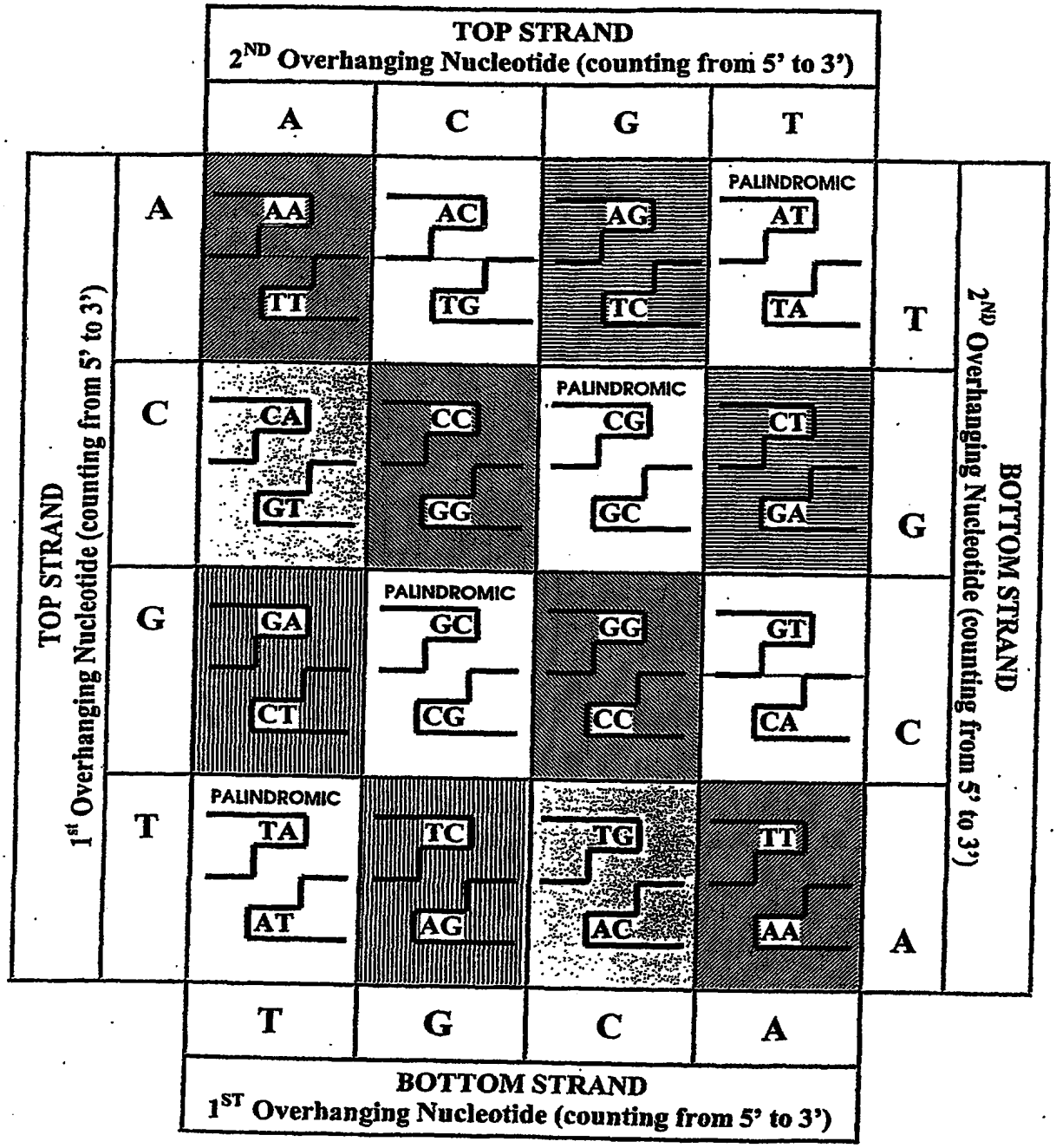


Figure 5. Unique Couplings Available Using a Two-Nucleotide 3' Overhang.

16 unique 3' overhangs can be formed using two-nucleotides. However, use of these 16 unique overhangs allows for the formation of only 6 unique couplings. Another 6 unique couplings are provided by the use 5' overhangs formed using two-nucleotides. Thus, a total of 12 unique couplings are provided by the combined use of 3' and 5' two-nucleotide overhangs. "Twin" couplings are marked in the same shading.



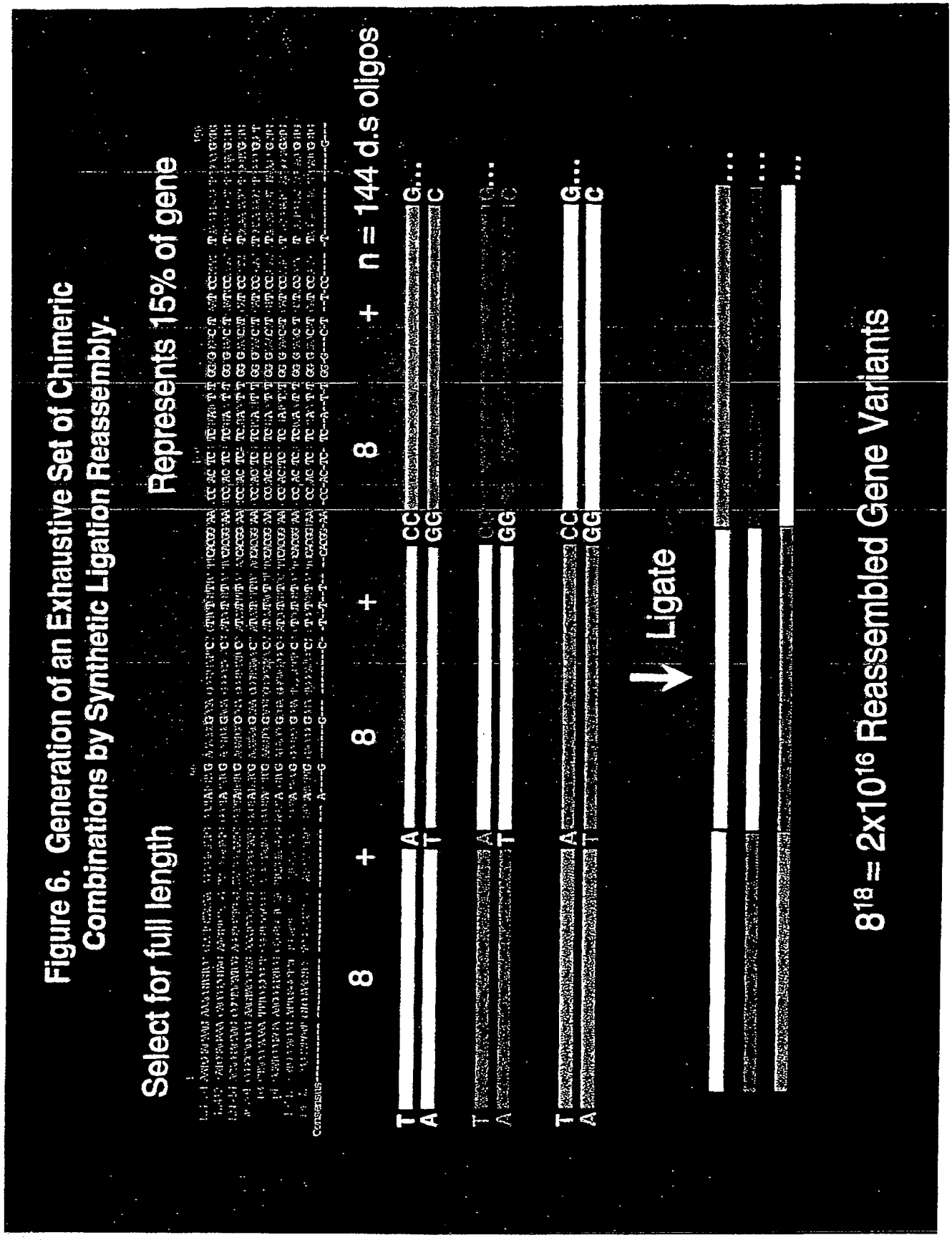


Figure 6. Generation of an Exhaustive Set of Chimeric Combinations by Synthetic Ligation Reassembly.
Select for full length
Represents 15% of gene
8 + 8 + 8 + n = 144 d.s oligos
Ligate
$8^{18} = 2x10^{16}$ Reassembled Gene Variants

Figure 6. Unique Couplings Available Using a Three-Nucleotide Overhang.

TOP STRAND – 2nd Overhanging Nucleotide (BOTTOM STRAND – Complementary Nucleotide)		TOP STRAND - 1ST Overhanging Nucleotide (BOTTOM STRAND – Complementary Nucleotide)				TOP STRAND – 3rd Overhanging Nucleotide (BOTTOM STRAND – Complementary Nucleotide)
		A	C	G	T	
A		AAA	CAA	GAA	TAA	A
		AAC	CAC	GAC	TAC	C
		AAG	CAG	GAG	TAG	G
		AAT	CAT	GAT	TAT	T
C		ACA	CCA	GCA	TCA	A
		ACC	CCC	GCC	TCC	C
		ACG	CCG	GCG	TCG	G
		ACT	CCT	GCT	TCT	T
G		AGA	CGA	GGA	TGA	A
		AGC	CGC	GGC	TGC	C
		AGG	CGG	GGG	TGG	G
		AGT	CGT	GGT	TGT	T
T		ATA	CTA	GTA	TTA	A
		ATC	CTC	GTC	TTC	C
		ATG	CTG	GTG	TTG	G
		ATT	CTT	GTT	TTT	T
		T	G	C	A	
		BOTTOM STRAND 1ST Overhanging Nucleotide (counting from 5' to 3')				

Figure 6. Unique Couplings Available Using a Three-Nucleotide 3' Overhang.

TOP STRAND				BOTTOM STRAND			
1st Base	2nd Base	3rd Base	Sequence 5'-XXX-3'	Sequence 3'-XXX-5'	Sequence 5'-XXX-3'	No.	Comments
A	A	A	AAA	TTT	TTT	1	
		C	AAC	TTG	GTT	2	
		G	AAG	TTC	CTT	3	
		T	AAT	TTA	ATT	4	
	C	A	ACA	TGT	TGT	5	
		C	ACC	TGG	GGT	6	
		G	ACG	TGC	CGT	7	
		T	ACT	TGA	AGT	8	
	G	A	AGA	TCT	TCT	9	
		C	AGC	TCG	GCT	10	
		G	AGG	TCC	CCT	11	
		T	AGT	TCA	ACT	12	
	T	A	ATA	TAT	TAT	13	
		C	ATC	TAG	GAT	14	
		G	ATG	TAC	CAT	15	
		T	ATT	TAA	AAT	16	
C	A	A	CAA	GTT	TTG	17	
		C	CAC	GTG	GTG	18	
		G	CAG	GTC	CTG	19	
		T	CAT	GTA	ATG	20	
	C	A	CCA	GGT	TGG	21	
		C	CCC	GGG	GGG	22	
		G	CCG	GGC	CGG	23	
		T	CCT	GGA	AGG	24	
	G	A	CGA	GCT	TCG	25	
		C	CGC	GCG		26	
		G	CGG	GCC		27	
		T	CGT	GCA		28	
	T	A	CTA	GAT		29	
		C	CTC	GAG		30	
		G	CTG	GAC		31	
		T	CTT	GAA		32	
G	A	A	GAA	CTT		33	
		C	GAC	CTG		34	
		G	GAG	CTC		35	
		T	GAT	CTA		36	
	C	A	GCA	CGT		37	
		C	GCC	CGG		38	
		G	GCG	CGC		39	
		T	GCT	CGA		40	
	G	A	GGA	CCT		41	
		C	GGC	CCG		42	
		G	GGG	CCC		43	
		T	GGT	CCA		44	
	T	A	GTA	CAT		45	
		C	GTC	CAG		46	
		G	GTG	CAC		47	
		T	GTT	CAA		48	
T	A	A	TAA	ATT		49	
		C	TAC	ATG		50	
		G	TAG	ATC		51	
		T	TAT	ATA		52	
	C	A	TCA	AGT		53	
		C	TCC	AGG		54	
		G	TCG	AGC		55	
		T	TCT	AGA		56	
	G	A	TGA	ACT		57	
		C	TGC	ACG		58	
		G	TGG	ACC		59	
		T	TGT	ACA		60	
	T	A	TTA	AAT		61	
		C	TTC	AAG		62	
		G	TTG	AAC		63	
		T	TTT	AAA		64	

Figure 7. Synthetic genes from oligos.

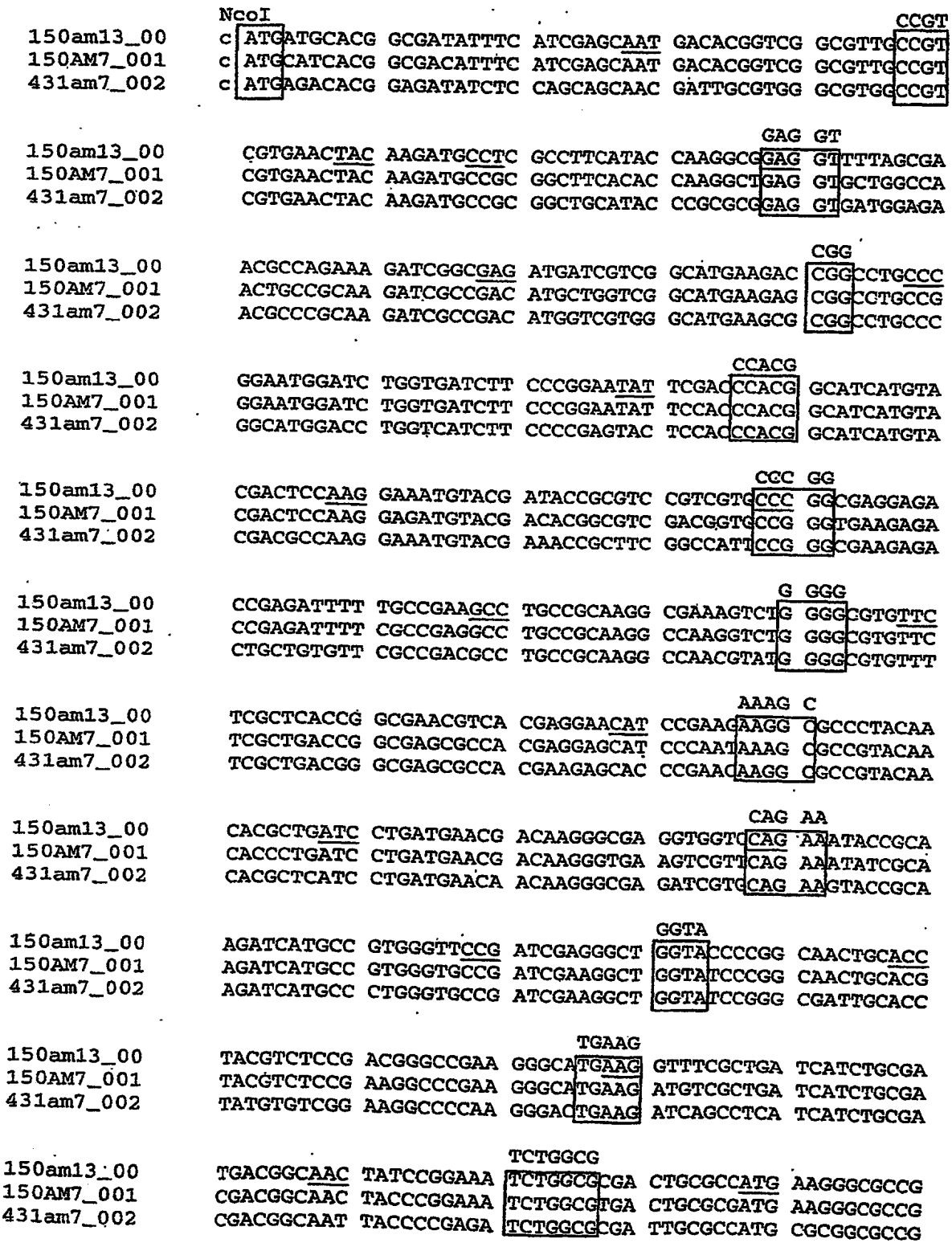


Figure 7 cont.

```
                                    CCAG
150am13_00      AGCTGATCGT GCGCTGCCAG GGCTACATGT ATCCGGCCAA GGACCAGCAG
150AM7_001      AACTGATCAT CCGCTGCCAG GGCTACATGT ATCCCGCCAA GGATCAGCAG
431am7_002      AGCTGATCGT GCGTTGCCAG GGATACATGT ACCCGGCCAA GGACCAGCAG

                                    GC
150am13_00      GTCATCATGG CGAAGGCGAT GGCGTGGGCG AATAATTGTT ACGTCGCGGT
150AM7_001      GTGCTGATGG CGAAAGCAAT GGCCTGGGCC AACAACGTTT ATGTCGCGGT
431am7_002      GTCATGGTGT CCAAGGCCAT GGCGTGGATG AACAACGTCT ACGTGGCGGT

                                GGGCTTCG
150am13_00      TTCCAATGCC GCGGGCTTCG ATGGCGTCTA TTCGTATTTC GGCCACTCGG
150AM7_001      CGCCAATGCC TCGGGCTTCG ACGGCGTCTA CTCGTATTTC GGCCATTCGG
431am7_002      GGCCAATGCC GCGGGCTTCG ACGGCGTGTA TTCCTACTTC GGCCATTCGG

                                TTCGA
150am13_00      CGATCATCGG CTTCGATGGC CGCACGCTCG GCGAATGCGG CGAGGAAGAA
150AM7_001      CGATCATCGG CTTCGACGGC CGTACCCTCG GCGAATGCGG CGAGGAGGAT
431am7_002      CCATCATCGG CTTCGACGGC CGCACGCTGG GCGAATGCGG TGAAGAAGAC

                              C AGTA
150am13_00      TACGGCATCC AGTATGCCCA GCTTTCGAAG ATGCTGATCC GCGACGCCCG
150AM7_001      TATGGCATCC AGTATGCCGC CATCTCCAAG TCGCTGATCC GCGACGCGCG
431am7_002      ATGGGCGTGC AGTACGCCGA GCTCTCCACC AGCCTGATCC GCGACGCGCG

                                CAATC
150am13_00      CCGCACCGGA CAATCGGAAA ACCATCTCTT CAAGCTGGTG CATCGTGGCT
150AM7_001      CCGCACCGGC CAATCGGAAA ACCATCTCTT CAAGCTGGTG CACCGTGGCT
431am7_002      CAAGAACATG CAGTCGCAGA ACCACTTGTT CAAGCTGGTG CACCGCGGCT

                                GATCAA
150am13_00      ACACCGGGTT GATCAACTCC GGCGAGGGCG ACCGCGGTCT CGCGGCCTGT
150AM7_001      ACACCGGCAT GATCAATTCC GGCGAGGGCG ACCGCGGTGT CGCGGCTTGC
431am7_002      ACACCGGCAA GATCAATTCC GGCGAAGAGG CCACCGGCGT CGCGGCATGC

                             TTA
150am13_00      CCTTATGAGT TCTACAACAA ATGGATCGCC GATCCGGAAG GCACCCGCGA
150AM7_001      CCGTATGATT TCTATTCGAA ATGGATCGCC GATCCCGAGG GTACACGCGA
431am7_002      CCGTACAACT TCTACGCCAA CTGGATCAAC GATCCGGAGG GCACGCGCAA

                           ATGGT
150am13_00      AATGGTCGAG TCCTTTACCC GGCCGACGGT GGGAACCGAT GAAGCGCCCA
150AM7_001      GATGGTGGAA TCCTTCACGC GTCCGACGGT GGGTGTGGAG GAATGCCCGA
431am7_002      GATGGTCGAA TCCTTCACCC GGTCCACCGT GGGCACGCCG GAGTGCCCCA

                          TCGAG
150am13_00      TCGAAGGCAT CCCGAACAAG GTCGCGGTGC ACCGCTGA   aagct
150AM7_001      TCGAGGGCAT TCCGAACAAG GCCACCACGC ACCGCTGA   aagct
431am7_002      TGGACGGCAT CCCCAACGAG GACGCCAAGC ACCGCTAG   aagct
                                                           HindIII
```

Figure 8. Nucleic acid building blocks for synthetic ligation gene reassembly.

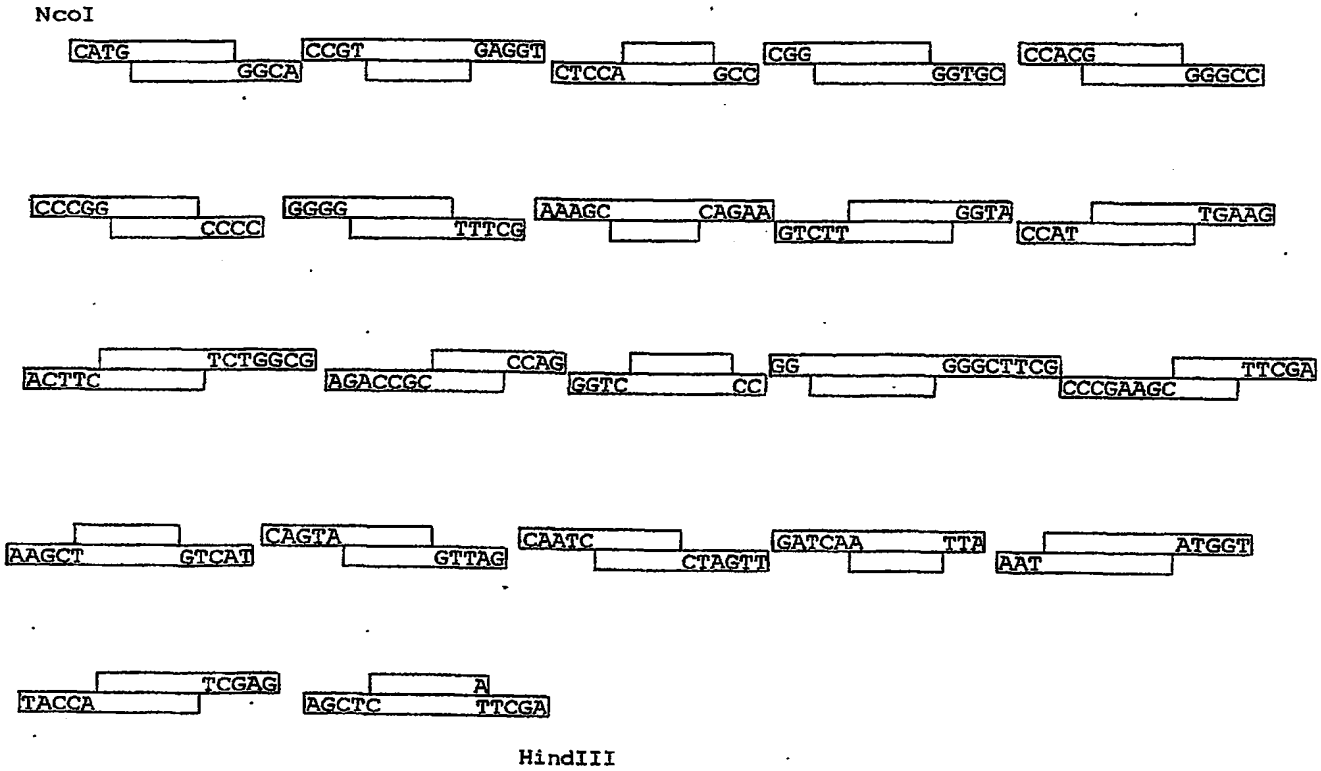


Figure 9. Addition of Introns by Synthetic Ligation Reassembly.

Figure 10. Ligation Reassembly Using Fewer Than All The Nucleotides Of An Overhang.

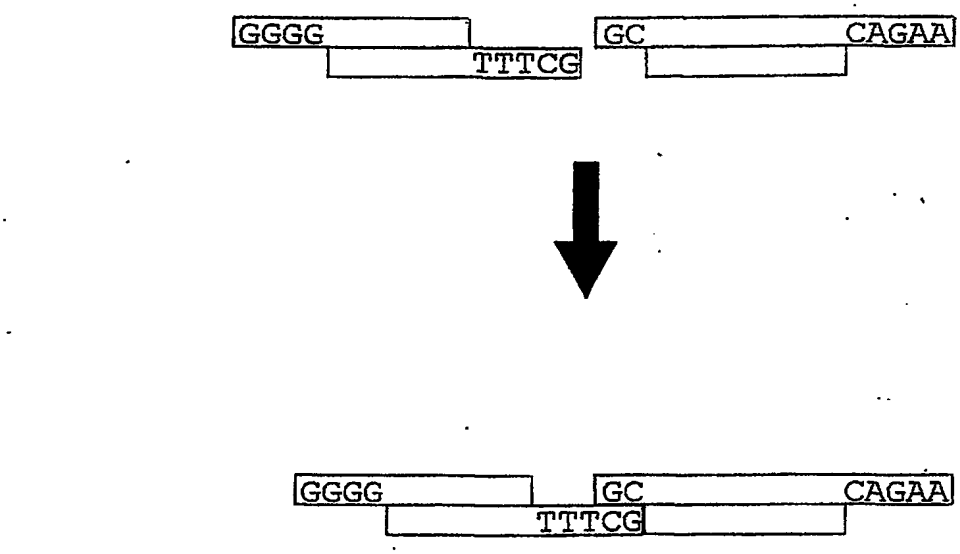
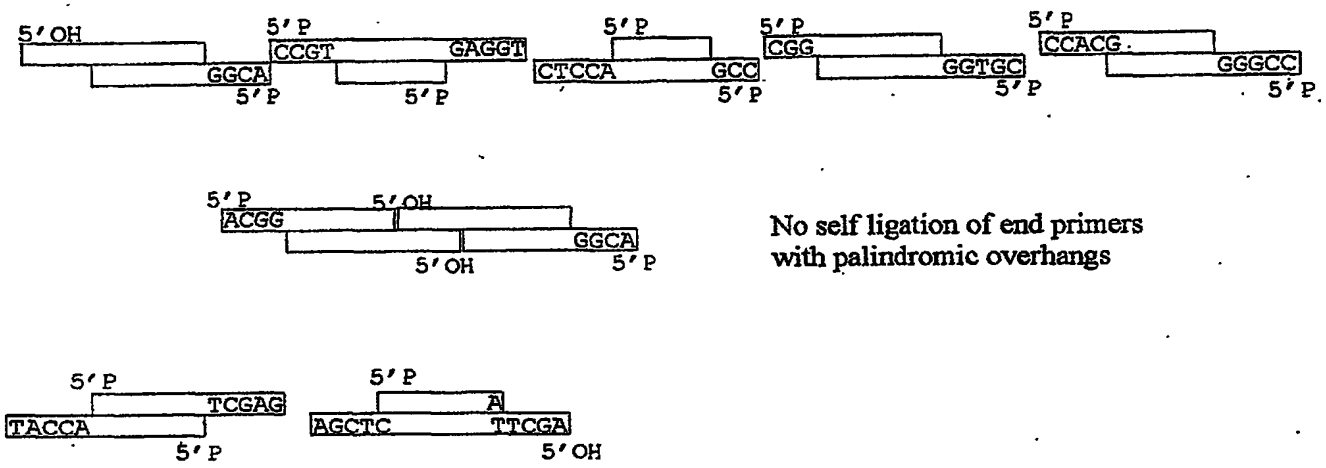


Figure 11. Avoidance of unwanted self-ligation in palindromic couplings.



Site-Directed Mutagenesis

Figure 12A

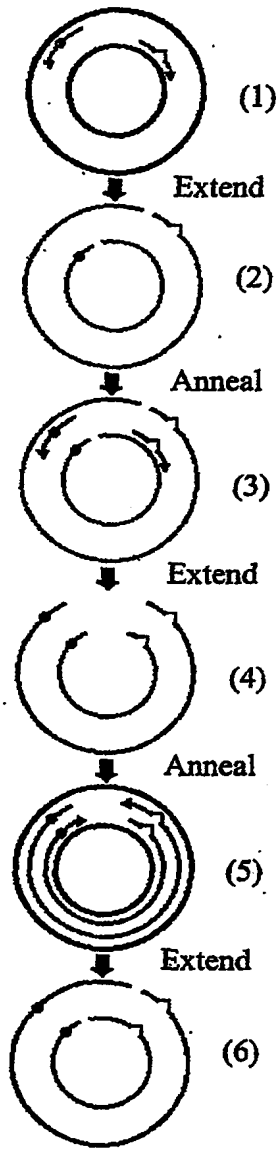


Figure 12B

Amplification products are comprised of the following molecular structures:

Site-Directed Mutagenesis

Figure 13A

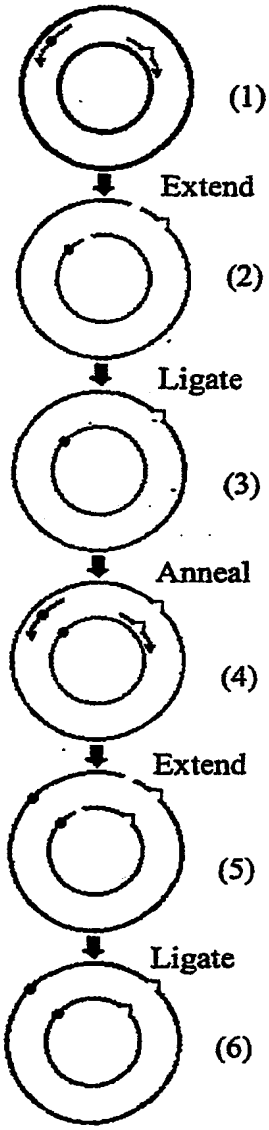


Figure 13B

Amplification products are comprised of the following molecular structures:

Molecule (A) Molecule (B)

Figure 14

Strategy for obtaining and using nucleic acid binding proteins that facilitate entry of genetic vaccines.

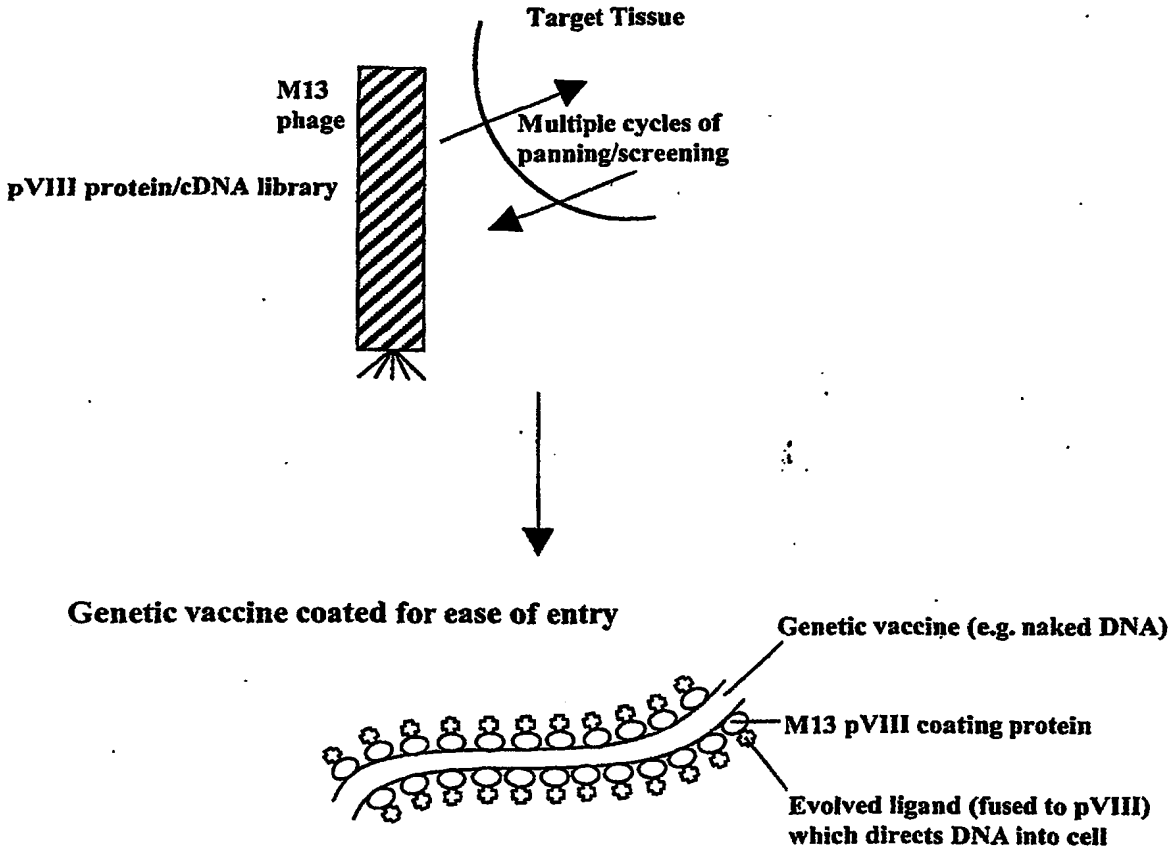


Figure 15

A schematic representation of a method for evolving a chimeric, multivalent antigen that has immunogenic regions from multiple antigens.